Survey for Spotted-Wing Drosophila (Diptera: Drosophilidae) in the Five-County Nursery Production Region of Middle Tennessee, USA

Authors: Addesso, Karla M., Oliver, Jason B., and O'Neal, Paul A.

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Survey for spotted-wing drosophila (Diptera: Drosophilidae) in the five-county nursery production region of middle Tennessee, USA

Karla M. Addesso, Jason B. Oliver, and Paul A. O'Neal*

Abstract

Through global trade, spotted-wing drosophila, *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae), a native of Southeast Asia, has spread to at least 3 other continents since 2008. Initial reports of the fly in new regions often are associated with damage in agriculture fields, but the fly may be present in the landscape for years before populations reach a level at which they become a pest in fruit production. In 2012, spotted wing drosophila was reported in blueberry fields in eastern Tennessee, USA, for the first time. In order to determine whether the fly was established in middle Tennessee, we conducted landscape surveys over 2 yr in the middle of the state's ornamental nursery industry where many fruit and ornamental hosts of the fly are grown. Red and yellow colored traps baited with yeast solution were placed in 17 locations of the 5-county nursery production region of middle Tennessee. Traps were monitored weekly for 8 wk in 2013 and 9 wk in 2014. Flies were caught at all 17 locations in 2013 and 16 of 17 locations in 2014. First activity was delayed 3 wk and total captures were 77% lower in 2014 relative to the previous year, likely due to high mortality of overwintering flies resulting from unusually cold winter temperatures in the region. No statistical differences were detected between trap colors in the landscape or between total captures of each sex, despite a trend for more female than male captures by the end of the season. Beginning Feb 2014, we also sampled weekly from a single yellow monitoring trap suspended within a plot of mixed-species dogwood trees (*Cornus* spp.; Caryophyllales: Cornaceae) for nearly 1 yr. Adult *D. suzukii* consistently were caught from late Jul until mid-Dec when the first frost occurred. Our surveys confirm that spotted wing drosophila is well established in the middle Tennessee nursery production region, despite no concurrent reports of damage by local small-fruit producers in the region.

Key Words: fruit fly; monitoring; landscape; invasive species

Resumen

A través del comercio mundial, la drosophila de ala manchada, *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae), una nativa del sudeste de Asia, se ha extendido a por lo menos 3 otros continentes desde el 2008. Los informes iniciales de esta mosca en nuevas regiones a menudo están asociados con daños en los campos de la agricultura, pero la mosca puede estar presente en el campo por años antes de que las poblaciones alcanzan un nivel en el que se convierten en una plaga en la producción de fruta. En 2012, se informó por primera vez de la drosophila de alas manchadas en campos de arándanos en el este del estado de Tennessee, EE.UU. Con el fin de determinar si se estableció la mosca en el centro de Tennessee, realizamos un sondeo de campo por 2 años en el centro de industria de viveros ornamentales del estado donde se cultivan muchas frutas y hospederos ornamentales de la mosca. Trampas de color amarillo y rojo y cebadas con una solución de levadura fueron colocadas en 17 localidades de la región de producción de viveros de 5 condados del centro de Tennessee. Las trampas fueron monitoreadas semanalmente durante 8 semanas en el 2013 y 9 semanas en el 2014. Las moscas fueron capturadas en 17 lugares en el 2013 y en 16 de los 17 lugares del 2014. El inicio de su actividad se retrasó 3 semanas y la captura total de moscas fue 77% más baja en el 2014 con relación al año anterior, probablemente por la alta mortalidad de las moscas hivernando debido a las temperaturas inusualmente frias de invierno en la región. No se detectaron diferencias estadísticas entre los colores de trampa en el campo o entre la captura total de cada sexo, a pesar de una tendencia de capturar más hembras que machos al final de la temporada. A partir de febrero del 2014, también mostramos semanalmente una sola trampa amarilla de monitoreo suspendida dentro de una parcela de especies de cornejos (*Cornus* spp.; Caryophyllales: Cornaceae) mixtas por casi 1 año. Los adultos de *D. suzukii* consistentemente fueron capturados desde el final de julio hasta el medio de diciembre, cuando sucede la primera helada. Nuestro sondeo confirma que la drosophila de alas manchadas está bien establecida en los viveros en la región de producción del centro de Tennessee, a pesar de que no hubo informes de daño concurrente por parte de productores de pequeñas frutas locales de la región.

Palabras Clave: mosca de la fruta; monitoreo; campo; especies invasivas

Spotted-wing drosophila, *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae), is an economically important agricultural pest of thin-skinned small fruits (Bolda et al. 2010; Goodhue et al. 2011). The species is seemingly well adapted as an invader, and it is likely that it may soon be distributed worldwide. Native to Southeast Asia, since 2008 it has invaded 41 U.S. states, Canada, Mexico, Spain, Italy, France, and southern Brazil (Hauser 2011; Lee et al. 2011; Deprá et al. 2014; National Agriculture Pest Information System 2015; Spotted Wing Drosophila Volunteer Monitoring Network 2015). Unlike most other *Drosophila* species, female *D. suzukii* possess a serrated ovipositor that enables them to pierce the skin of healthy, ripening fruit (Atallah et al. 2014). Fruit are rendered unsalable as they become discolored

Tennessee State University, Agricultural and Environmental Sciences Department, Nashville, Tennessee 37209, USA
*Corresponding author; E-mail: kaddesso@tnstate.edu

and collapse from larval feeding and incur secondary microbial infections (Walsh et al. 2011). Especially troubling is that *D. suzukii* utilizes a wide range of hosts. In addition to commercial fruit crops, the species attacks ornamental and wild species (Lee et al. 2015). Hosts include several members of the family Rosaceae (Rosales)—including *Prunus* (cherries, peaches, plums, and apricots), *Fragaria ananassa* (Weston) Duchesne ex Rozier (strawberry), and *Rubus* (blackberries and raspberries) —, species of *Vaccinium* (blueberry; Ericales: Ericaceae), *Vitis* (grape; Vitales, Vitaceae), *Lonicera* (honeysuckle; Dipsacales: Caprifoliaceae), and *Cornus* (dogwoods; Caryophyllales: Cornaceae), and *Sambucus nigra* L. (black elder; Dipsacales: Adoxaceae) (Cini et al. 2012). Much of the distribution data in the U.S. and Europe comes from trap captures near fruit production areas or first-hand accounts of infestations (Lee et al. 2011; Burrack et al. 2012). Despite vigilant monitoring of crop fields, there has been relatively little effort to assess *D. suzukii* populations in surrounding landscapes. The species' wide host range suggests wild fruit in natural habitats and ornamentals in anthropic environments could serve as reservoirs for *D. suzukii* populations and may provide refuge between crop plantings (Klick et al. 2015).

A major component of recent *D. suzukii* research has been to develop management tools that better predict the timing and severity of infestations. One element of this research has been whether a color preference exists that can improve the efficiency of early season traps, which are important in establishing economic thresholds. Cage experiments offering choices between cards of 9 colors showed that darker colors attracted *D. suzukii* more than lighter colors (Basoalto et al. 2013). Significantly more flies landed first on red, burgundy, and black cards than white, yellow, and light blue cards. Color may be a crucial factor of trap effectiveness in production fields where high densities of ripening fruits can compete with traps. However, it is unclear how important such cues are to monitoring in natural landscapes where hosts are more diverse and patchily distributed than in monoculture production.

The 5-county region of middle Tennessee (Warren, Franklin, Grundy, Coffee, and DeKalb Counties) is a major center of woody ornamental production with approximately 560 nurseries contributing over $259 million to Tennessee's economy (Tennessee Deptartment of Agriculture 2011). Hosts of *D. suzukii* such as kousa dogwood, ornamental cherry, blueberry, blackberry, and other susceptible species are commonly cultivated in area nurseries. Additionally, wild hosts such as blackberries and dogwoods are ubiquitous in the region. In 2012, Unicoi County, which forms the state border with North Carolina, was the first county in Tennessee with a *D. suzukii* detection (Burrack et al. 2012, Spotted Wing Drosophila Volunteer Monitoring Network 2015). No other records of *D. suzukii* were recorded in Tennessee until the authors reported a catch in Warren County in Dec 2012 (Spotted Wing Drosophila Volunteer Monitoring Network 2015). A monitoring survey was conducted in order to determine the extent of *D. suzukii* establishment in the middle Tennessee landscape, specifically in the 5-county nursery production region, where concerns were raised about the fly's presence in ornamental hosts.

Materials and Methods

To test for *D. suzukii*'s presence in the nursery production region of middle Tennessee, 2 monitoring studies were performed. In the 1st, flies were trapped along roadways in the 5-county area to determine the extent of *D. suzukii*'s presence in the nursery production region. We used red and yellow banded traps (described below) to test for a color preference in *D. suzukii* inhabiting municipal environments. The 2nd was a year-long study conducted within a plot of mixed species of dogwoods to monitor a *D. suzukii* population in an ornamental production setting.

FIVE-COUNTY SURVEY

Thirty-four traps were deployed at 17 locations (2 traps per location) during each of the summers of 2013 and 2014, with 5 trap locations in Warren County, where the majority of state nursery production occurs, and 3 trap locations in each of the neighboring counties DeKalb, Coffee, Franklin, and Grundy. Fifteen trap locations, 3 per county, were situated in public-access areas along roadways and were spaced approximately 12 to 16 km apart. The 2 remaining Warren County traps were located on the grounds of Tennessee State University's Otis L. Floyd Nursery Research Center (TSU Research Center) and at the Rock Island State Park Bluff Trail trailhead.

Each trap location had 2 traps constructed from translucent 946 mL (32 oz) containers (Safe Lock Polypropylene Containers, U.S. Plastic Corporation, Lima, Ohio, USA), with four 1 cm access holes drilled around the top third of the container. A strip of either red (*L*a*b*, 45.59*42.42*23.65) or yellow (*L*a*b*, 76.08*6.07*62.32) 2.5 cm (1 in) wide vinyl tape (3M, St. Paul, Minnesota, USA) was wrapped around the circumference of each container forming a band just below the access holes. Vinyl tape rolls used for banding were scanned using a portable Konica Minolta CM-2600d spectrophotometer and SpectraMagic (Version 3.61 Release No. 2; Konica Minolta, Tokyo, Japan) software and were defined using the Hunter 1948 color space dimensions defined as *L*a*b* (*L* = lightness, *a* and *b* = the color opponent dimensions). The spectrophotometer unit was set in observer illuminant Daylight 65 (i.e., average daylight including UV radiation at 6500k), observer angle at 10° (CIE1964), specular component included (SCI), and ultraviolet (UV) at 100% (i.e., illumination contains all UV components of the Xenon light source). The red and yellow traps were placed approximately 5 m apart. Traps were suspended from low-hanging branches when available or from trap rods 1.5 m above the ground. When possible, traps were placed in locations with nearby wild host plants (e.g., *Rubus* species) to increase the likelihood of catching *D. suzukii*. Traps were baited with 250 mL yeast mixture (3.8 L water, 56 g dry active yeast [Fisher Scientific, Pittsburgh, Pennsylvania, USA], 170 g (6 oz) sucrose (Bio-Serv, Flemington, New Jersey, USA), and 1 mL Sparkleen dish detergent (Fisherbrand, Pittsburgh, Pennsylvania, USA) to disrupt water surface tension). In 2013, traps were placed in the field on 3 Jun and were serviced weekly for 8 wk from 10 Jun to 31 Jul. In 2014, traps were deployed on 4 Jun in the same locations as in 2013 and were serviced weekly for 9 wk from 11 Jun to 9 Aug. Samples were strained with a paint filter and returned to the laboratory for processing. Following sample collection, each container received fresh bait and the traps were re-randomized to reduce uneven exposure to local environmental conditions. *Drosophila suzukii* specimens were identified and sexed using the Oregon State University identification key (Dreves & Langellotto-Rhodaback 2011).

CORNUS PLOT MONITORING

On 17 Feb 2014, we began monitoring a block of approximately 300 *Cornus* trees consisting of 5 species (*C. kousa* F. Buerger *ex* Hance, *C. florida* L., *C. capitata* Wall. ex Roxb., *C. controversa* Hemsl. ex Prain, and *C. mas* L.), most of which were *C. florida* and *C. kousa*, on the grounds of the TSU Nursery Research Center in McMinnville, Warren County, Tennessee, USA. *Drosophila suzukii* adults had been observed in the plot and reared from fruit the previous summer. A single yellow banded trap was placed in the middle row at the center of the *Cornus* plot and monitored using the trap and sampling protocol described earlier.

STATISTICAL ANALYSES

For 5-county surveys, *D. suzukii* count data were analyzed separately for each year using a repeated measures generalized linear interactive model (GLIM) (PROC GENMOD; SAS Institute, Cary, North Carolina, USA) with a log link and assuming a negative binomial distribution (Agresti 2002). County, sex, and trap color were assigned as independent variables. Interaction terms were tested for significance and when non-significant, were dropped from the model. Pooled weekly counts were analyzed with the same model and distribution. To test pair-wise comparisons, treatment least squares means were separated by least significant difference ($P < 0.05$).

Results

FIVE-COUNTY SURVEY

2013 Trapping

In 2013, *D. suzukii* adults were captured in all 5 counties during the 1st week of collection (12 Jun) and at all 17 sites over the course of the study. Trap captures were significantly affected by week ($\chi^2_{(7)} = 347.34$, $P < 0.0001$; Table 1), county ($\chi^2_{(4)} = 20.46$, $P = 0.0004$; Table 2), and sex ($\chi^2_{(1)} = 5.52$, $P = 0.0188$) with significant week*county ($\chi^2_{(28)} = 55.30$, $P = 0.0016$), week*sex $\chi^2_{(8)} = 15.77$, $P = 0.0273$; Fig. 1), and sex*county ($\chi^2_{(4)} = 14.62$, $P = 0.0055$) interactions. Trap color did not affect trap catches ($\chi^2_{(1)} = 0.09$, $P = 0.7671$; Fig. 2). Weekly catch totals increased steadily through week 6 from 41 flies on 12 Jun to 453 flies on 17 Jul (Table 1). On 24 Jul and 31 Jul, catches increased dramatically, totaling 28% and 53%, respectively, of flies captured. Altogether, 6,485 flies were captured in 2013 over the 8 wk monitoring period.

2014 Trapping

Trap captures were significantly affected by week ($\chi^2_{(8)} = 429.05$, $P < 0.0001$; Table 1) and county ($\chi^2_{(4)} = 24.86$, $P < 0.0001$; Table 2) with significant week*county ($\chi^2_{(32)} = 64.11$, $P = 0.0006$), week*sex ($\chi^2_{(8)} = 16.84$, $P = 0.0319$; Fig. 1) and sex*county ($\chi^2_{(4)} = 12.29$, $P = 0.0153$) interactions. Trap color did not affect trap catches ($\chi^2_{(1)} = 0.01$, $P = 0.9068$; Fig. 2), nor did sex ($\chi^2_{(1)} = 0.94$, $P = 0.3328$). In 2014, *D. suzukii* captures were delayed and markedly lower relative to the 2013 capture data. *Drosophila suzukii* were not captured until week 4 (3 Jul) of trapping, and the species was not captured in all 5 counties in the same week until week 6 (17 Jul). Total captures were minimal during the first 6 wk of trapping, with no flies caught in the first 3 wk and only 18 flies in total caught in week 6 (Table 1). Beginning in week 7 (24 Jul), weekly total captures increased rapidly until the end of the study in week 9 (7 Aug).

Table 1. Total and average trap captures **± standard error** of *Drosophila suzukii* by week for both study years.

	2013		2014	
Week	Total	Trap Avg **± SE**	Total	Trap Avg **± SE**
12-Jun	41	1.21 ± 0.31a	0	0 ± 0a
19-Jun	67	1.97 ± 0.51a	0	0 ± 0a
26-Jun	152	4.47 ± 1.35b	0	0 ± 0a
3-Jul	261	7.68 ± 4.04bc	2	0.06 ± 0.04b
10-Jul	283	8.32 ± 1.80cd	5	0.15 ± 0.09b
17-Jul	453	13.32 ± 3.83d	18	0.53 ± 0.17b
24-Jul	1,824	57.00 ± 12.15e	220	6.47 ± 3.33c
31-Jul	3,404	106.38 ± 18.94f	486	14.29 ± 4.79d
7-Aug			732	21.53 ± 8.27e
Grand total	6,485		1,463	

Lowercase letters denote significant differences by pair-wise LSMeans comparison in the negative binomial regression analysis (PROC GENMOD).

Table 2. Total and average trap captures ± standard error of *Drosophila suzukii* by county for both study years.

	2013		2014	
County	Total	Trap Avg ± SE	Total	Trap Avg ± SE
Franklin	1,750	36.46 ± 11.18a	733	13.57 ± 6.15a
DeKalb	1,416	30.78 ± 9.51a	276	5.11 ± 1.66b
Warren	1,912	23.90 ± 6.55a	281	3.12 ± 1.00c
Grundy	686	14.91 ± 5.13b	126	2.33 ± 0.75c
Coffee	725	15.10 ± 4.76b	47	0.87 + 0.51d

Warren County sites = 5, remaining counties = 3; 2013 weeks = 8, 2014 weeks = 9. Lowercase letters denote significant differences by pair-wise LSMeans comparison in the negative binomial regression analysis (PROC GENMOD).

CORNUS PLOT MONITORING

Trap catch was evaluated 46 times from 24 Feb 2014 to 12 Feb 2015. The emergence of the *D. suzukii* population in the *Cornus* dogwood plot was comparable with the population in the surrounding landscape sampled in the 5-county survey. The first observed fly was a male caught on 18 Jun 2014. However, there were no additional flies captured until 24 Jul. After 24 Jul, flies were caught every week, uninterrupted, until 19 Nov (Fig. 3). Despite occasional subzero nighttime temperatures, *D. suzukii* adults were still caught at relatively low numbers for 3 wk in Dec and again on 13 Jan 2015.

The *Cornus* plot trap regularly captured more flies than were captured in the 5-county survey. On only 2 occasions during the 9 wk trapping period of the 2014 five-county survey did a trap capture ≥ 100 flies. In contrast, the *Cornus* plot caught between 100 and 207 flies each week for 7 wk beginning on 20 Aug (Fig. 3). At the end of that 7 wk period, beginning on 8 Oct, trap captures increased considerably, with 5 of the next 6 wk having capture ranges between 377 and 857 flies. Activity decreased dramatically with the onset of overnight freezing temperatures.

Discussion

The results of the 5-county survey confirmed that *D. suzukii* is present and widespread in middle Tennessee. The species was captured at all 17 of the trapping sites in 2013 and at 16 of the 17 sites in 2014, indicating it is capable of establishing in a heterogeneous landscape of natural and managed habitats, in anthropic environments, and in areas with ornamental production. Adult capture in a mixed-species *Cornus* site during the months when dogwood berries were present also provided evidence for *D. suzukii*'s capability to reproduce in an ornamental production setting where fruiting hosts are present.

The invasion of the Americas and Europe by *D. suzukii* has compelled research directed at refining trap design to better estimate when population thresholds call for pest management. Caged choice bioassays showed *D. suzukii* was more likely to be first attracted to black, red, and burgundy cards than to yellow cards (Basoalto et al. 2013). However, field studies, including the current one, indicate *D. suzukii*'s preference for red over yellow does not extend to the field. In an expansive study of 16 North American sites and 9 crop types, Lee et al. (2013) found no preference between yellow and red traps, though yellow traps did outperform black, white, and clear traps. When the analysis was limited to traps sus-

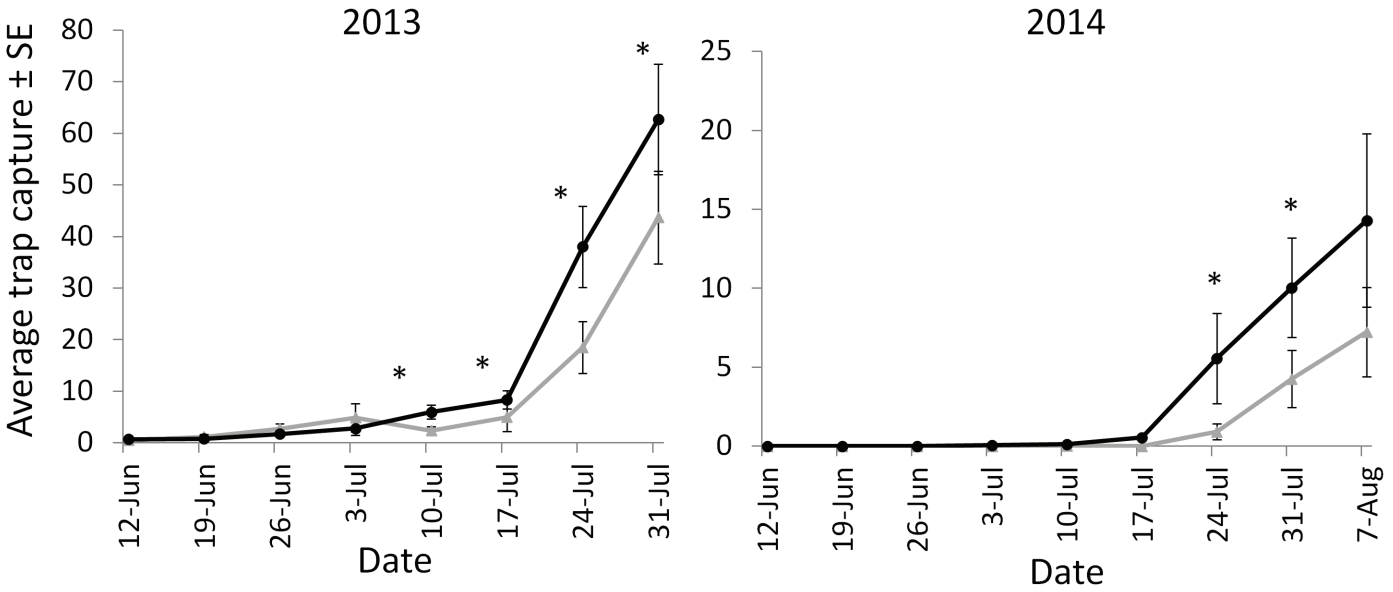

Fig. 1. Average trap captures of *Drosophila suzukii* males (gray triangles) and females (black circles) by week in 2013 and 2014. Weeks with an asterisk above them indicate significant differences ($P < 0.05$) between the sexes by pair-wise LSMeans comparison in the negative binomial regression analysis (PROC GENMOD).

pended among crops with red fruit, red traps were found to capture more than white traps, but there was still no statistical difference detected between red, black, or yellow traps (Lee et al. 2013). Further, the results from our survey of roadways and natural areas in the 5-county nursery region found no evidence for a preference for red traps over yellow traps, indicating the non-preference is consistent across multiple landscape types. These field tests suggest adults respond similarly to traps incorporating colors that resemble the colors host fruits exhibit during development (i.e., throughout ripening), and the behavior may not depend on host density. The limited improvement of trap efficiency through color manipulation indicates refining olfactory cues that mimic rotting fruit may be a more promising line of research.

The first sustained activity of *D. suzukii* in central Tennessee was delayed in 2014 relative to 2013. In 2014, no flies were captured until 3 Jul, a delay of at least 3 wk from the observed onset of activity during 2013. These delays in flight onset during summer 2014 were likely due, in part, to the harsh conditions the preceding winter. Data from a weather station on the grounds of the TSU Research Center indicated 3 cold snaps in Jan 2014 during which the average daily temperatures ranged between −12.9 °C and −7.4 °C, temperatures well below the region's historical average of 2.4 °C for Jan (National Climatic Data Center 2015). Other monitoring studies, including the current one, have reported trap captures often reach their peak in the weeks leading up to the first 0 °C day (Dalton et al. 2011; Harris et al. 2014). Additionally, during this study we caught

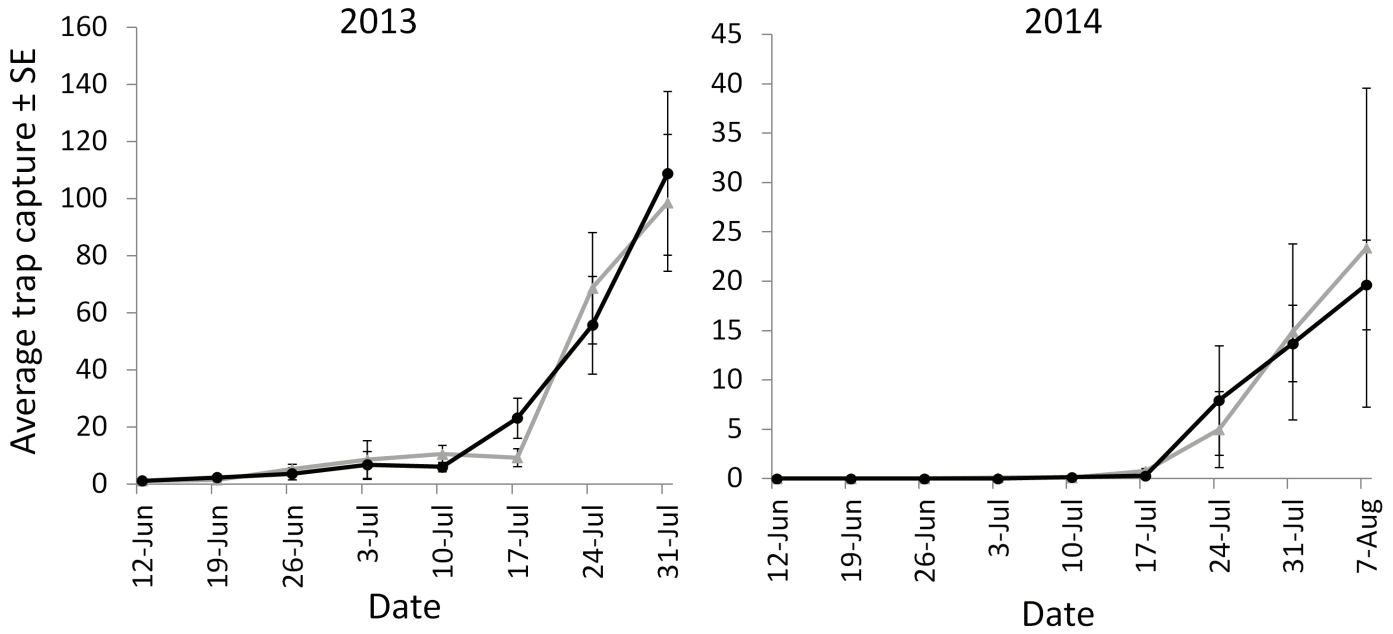

Fig. 2. Average trap captures of *Drosophila suzukii* in red (black circles) and yellow traps (gray triangles) by week in 2013 and 2014. No significant differences ($P > 0.05$) were found between the colors by pair-wise LSMeans comparison in the negative binomial regression analysis (PROC GENMOD).

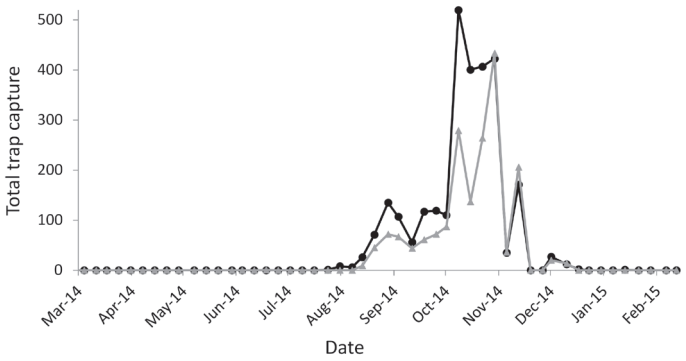

Fig. 3. Adult *Drosophila suzukii* males (gray triangles) and females (black circles) captured from a yeast-baited deli cup trap in a plot of mixed *Cornus* species at the Otis L. Floyd Nursery Research Center during 2014 and 2015.

adults in both Dec and Jan during days when ambient temperatures were above the threshold for flight (12.8 °C), indicating the species may become quiescent at the onset of low temperatures and delay diapause until later in the season or fail to enter a diapause at all. Dalton et al. (2011) found that a majority of non-diapausing adult and pupal *D. suzukii* exposed to a constant temperature of 1 °C died within 8 d and 100% were dead by day 17. Although the Jan 2014 cold snaps lasted on average only 2 d, the mean daily temperatures were ≥ 8 °C lower, suggesting mortality may have been comparable and the unusually cold weather may have contributed to the delay in first capture. These unexpected winter adult occurrences suggest the species may be adapting to the variation in winter conditions across the geographical range of its invasion. As the species continues to spread, it is likely to face selective pressure to adapt diapause phenology, endure conditions of varying severity and length, and alter refuge-seeking behavior with colonization of new ecosystem types. Increased research into the species' overwintering behaviors, physiology, and cold tolerance may provide tools to growers that aid them in predicting dates of first activity and severity of infestation based on climatic conditions.

The extent of *D. suzukii*'s expansion over the past decade indicates the invasions likely have been assisted through the movement of infested fruits via human trade. The difficulty in detecting a recent attack on fruit, in conjunction with poor sanitation of rejected product, likely contributes to the inadvertent transfer of *D. suzukii* to new markets. Ornamentals decorating private residences and businesses in urban and rural areas may, along with wild fruits, serve as semi-ubiquitous hosts that bolster newly introduced populations and act as corridors to nearby fruit production areas. A study on *D. suzukii* in the Forest of Compiègne in France estimated wild *Prunus serotina* Ehrh. alone may sustain 8.36 billion adults (Poyet et al. 2014). In a monitoring study by Harris et al. (2014) in a mixed-fruit production system, *D. suzukii* captures declined in the winter months in deciduous species and increased in traps in a plot of year-round fruiting *Citrus* species and in traps suspended in bordering evergreen trees. These observations suggest *D. suzukii* will seek alternative hosts or refuge in permanent foliage and/or other structures at the onset of winter. Our finding that *D. suzukii* was present throughout the 5-county nursery region indicates the species is capable of finding viable hosts to allow its spread across a landscape that includes ornamental production fields.

Given the potential for economic losses, most of the *D. suzukii* research has been directed at management in fruit production. As the pest continues to expand its invasion across the U.S. and Europe, more attention should be paid to its ecology in natural environments. We suggest a better understanding of the species' use of ornamental and wild hosts can improve management in fruit production by providing information on local population reservoirs required to predict the severity of agricultural infestations.

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